

Founder *BRCA1* and *BRCA2* Mutations in French Canadian Breast and Ovarian Cancer Families

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Summary

We have identified four mutations in each of the breast cancer-susceptibility genes, *BRCA1* and *BRCA2*, in French Canadian breast cancer and breast/ovarian cancer families from Quebec. To identify founder effects, we examined independently ascertained French Canadian cancer families for the distribution of these eight mutations. Mutations were found in 41 of 97 families. Six of eight mutations were observed at least twice. The *BRCA1* C4446T mutation was the most common mutation found, followed by the *BRCA2* 8765delAG mutation. Together, these mutations were found in 28 of 41 families identified to have a mutation. The odds of detection of any of the four *BRCA1* mutations was $18.7 \times$ greater if one or more cases of ovarian cancer were also present in the family. The odds of detection of any of the four *BRCA2* mutations was $5.3 \times$ greater if there were at least five cases of breast cancer in the family. Interestingly, the presence of a breast cancer case <36 years of age was strongly predictive of the presence of any of the eight mutations screened. Carriers of the same mutation, from different families, shared similar haplotypes, indicating that the mutant alleles were likely to be identical by descent for a mutation in the founder population. The identification of common *BRCA1* and *BRCA2* mutations will facilitate carrier detection in French Canadian breast cancer and breast/ovarian cancer families.

Introduction

Germ-line mutations in the breast cancer-susceptibility genes, *BRCA1* and *BRCA2*, account for a large proportion of hereditary breast cancer and breast/ovarian cancer families. Screening for mutations has been hampered by the large size of the *BRCA1* and *BRCA2* genes (Miki et al. 1994; Wooster et al. 1995; Tavtigian et al. 1996), the frequent occurrence of unique mutations (Breast Cancer Information Core database), and putative "regulatory mutations" (Serova et al. 1996). However, within defined ethnic groups, specific relatively frequent mutations have been identified. Three founder mutations have been identified in the Ashkenazi Jewish families of eastern European ancestry (Friedman et al. 1995; Struwing et al. 1995b; Tonin et al. 1995; Berman et al. 1996a, 1996b; Fitzgerald et al. 1996; Neuhausen et al. 1996a). Specific mutations also have been identified in Icelandic, Swedish, British, Austrian, Dutch, Belgian, Russian, and Hungarian families, and haplotype analysis has provided evidence for founder effects in these ethnic groups (Simard et al. 1994; Gayther et al. 1995; Shattuck-Eidens et al. 1995; Johannesdottir et al. 1996; Johannsson et al. 1996; Wagner et al. 1996; Gayther et al. 1997; Peelen et al. 1997; Ramus et al. 1997; Shattuck-Eidens et al. 1997). The presence of founder effects, leading to reduced genetic heterogeneity, facilitates carrier detection and genetic counseling, for certain well-defined populations.

The French colonization of the province of Quebec began in 1608 and continued until 1760 (Charbonneau and Robert 1987). An estimated 8,000–10,000 French migrants established themselves permanently in the province (Charbonneau et al. 1987; Bouchard et al. 1988). Many of the hereditary disorders in the French Canadian population show evidence of founder effects (De Braekeleer and Dao 1994a, 1994b). For example, five specific mutations in the low-density lipoprotein re-

Received March 31, 1998; accepted for publication September 8, 1998; electronically published October 23, 1998.

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0002-9297/98/6305-0011\$02.00

Table 1**Primer Sequences Used to Detect *BRCA1* and *BRCA2* Mutations in French Canadian Families**

Gene and Exon	Mutation	Effect	Annealing Temperature	Primer Pair	Reference
<i>BRCA1</i> :					
11	C2598A	Thr826Lys	60	Forward: 5'-TAT GGC ACT CAG GAA AGT ATC TCG-3' Reverse: 5'-GCG CTT TGA AAC CTT GAA TGT AT-3'	Simard et al. (1994)
11	2953del3+C	950ter	55	Forward: 5'-TGC AGG CTT TCC TGT GGT TG-3' Reverse: 5'-GGC TAA TTG TGC TCA CTG TAC TTG G-3'	Durocher et al. (1996)
11	3768insA	1218ter	57	Forward: 5'-GAG TCC TAG CCC TTT CAC CCA TAC-3' Reverse: 5'-GTG ATG TTC CTG AGA TGC CTT TG-3'	This study
13	C4446T	Arg1443ter	55	Forward: 5'-AAT GGA AAG CTT CTC AAA GTA-3' Reverse: 5'-ATG TTG GAG CTA GGT CCT TAC-3'	This study
<i>BRCA2</i> :					
11	2816insA	880ter	55	Forward: 5'-CCC ATG GAA AAG AAT CAA GAT G-3' Reverse: 5'-GTT CCT TAG TAT TCC TAA AGC-3'	This study
11	G6085T	Glu1953ter	55	Forward: 5'-AAG TGA AAG ACA TAT TTA CAG ACA G-3' Reverse: 5'-TAT GAA GCT TCC CTA TAC T-3'	This study
11	6503delTT	2099ter	55	Forward: 5'-AAG TAA CGA ACA TTC AGA CC-3' Reverse: 5'-CTG GGT TTC TCT TAT CAA CAG CA-3'	This study
20	8765delAG	2867ter	55	Forward: 5'-CAC TGT GCC TGG CCT GAT AC-3' Reverse: 5'-AGT CTC TAA GAC TTT GTT CTC-3'	Phelan et al. (1996)

ceptor gene account for 76% of familial hypercholesterolemia (Davignon and Roy 1993). Founder effects also have been described for β -thalassemia (De Braekeleer and Dao 1993), cytochrome C oxidase deficiency (Morin et al. 1993), cystic fibrosis (Rozen et al. 1992), pseudo vitamin D-deficiency rickets (Labuda et al. 1996), oculopharyngeal muscular dystrophy (Brais et al. 1995; Stajich et al. 1996), a severe form of peripheral neuropathy and agenesis of the corpus callosum (Causaubon et al. 1996), and autosomal recessive spastic ataxia of Charlevoix-Saguenay (A. Richter, J. Rioux, J.-P. Bouchard, J. Mercier, J. Mathieu, J. Poirier, D. Julien, G. Gyapay, J. Weissenbach, T. J. Hudson, K. Morgan, and S. B. Melançon, unpublished data). We have identified four mutations in each of the breast cancer-susceptibility genes, in French Canadian breast cancer and breast/ovarian cancer families; three mutations have been reported elsewhere (Simard et al. 1994; Durocher et al. 1996; Phelan et al. 1996), and five additional mutations are reported in this article. Given the expectation of a founder effect, we examined independently ascertained French Canadian breast cancer and breast/ovarian cancer families for the distribution of these eight mutations and associated haplotypes.

Families, Material, and Methods

Families

The index cases for mutation analysis represent 97 independently ascertained families that were recruited at the following centers: the Hereditary Cancer Clinics of McGill University, Montreal (35 families); the Breast

Clinic of the Centre Recherche CHUM Campus Hôtel Dieu (29 families) and the Gynecology and Oncology Clinic of Centre Recherche CHUM Campus Notre Dame, Montreal (24 families); and the Hereditary Cancer Clinics affiliated with the University of Toronto, Toronto (9 families). Four families (179, 290, PG1940, and hd13) have been described elsewhere (Simard et al. 1994; Durocher et al. 1996; Phelan et al. 1996). Each family had at least three cases of female breast cancer (diagnosed at <65 years of age), epithelial ovarian cancer, or male breast cancer; and two affected individuals in each family were either the first-, second-, or third-degree relatives of the index case for the mutation analysis. The families had cases of breast cancer only ($n = 48$) or breast and ovarian cancer ($n = 49$). Eight families had one or more individuals with male breast cancer. The cancer sites for at least three of the affected individuals in each family were confirmed by a review of pathology reports or death certificates. The index case was asked about her French Canadian ancestry and the location of settlement in Canada of her grandparents. Cancers at other sites also were recorded and, when possible, confirmed in the same manner. The study was approved by the appropriate institutional review boards, and informed consent was obtained from each participating individual.

The Panel of French Canadian *BRCA1* and *BRCA2* Mutations

The panel of *BRCA1* and *BRCA2* mutations used to screen the index cases comprises three mutations that have been reported elsewhere (Simard et al. 1994; Du-

rocher et al. 1996; Phelan et al. 1996) and five additional mutations not reported previously in French Canadian families (table 1). The *BRCA1* C4446T mutation was identified in family 482 by routine screening for mutations, by use of SSCP analysis, as described below. The mutations 3768insA and 6503delTT were identified in families W9072 and W98074, respectively, by use of a protein-truncation test (PTT) assay of exon 11 for *BRCA1* and of exons 10 and 11 for *BRCA2*, essentially as described by Hogervost et al. (1995). The primer sequences used in the PTT assays were obtained from the Breast Cancer Information Core database. PTT was performed by use of the TNT reticulocyte lysate system (Promega), by incorporation of ^{35}S -methionine/cysteine (New England Nuclear) for protein detection. The *BRCA1* 2816insA and *BRCA2* G6085T mutations were identified in families 107 and 787, respectively, by use of the SSCP assay described by Phelan et al. (1996).

Screening for the Panel of French Canadian BRCA1 and BRCA2 Mutations

The primers used to detect the mutations are given in table 1. PCR was performed in 12.5- μl volumes containing 50 ng genomic DNA; 1 \times PCR buffer (Pharmacia); 200 μM each of dCTP, dGTP, and dTTP; 10 μM dATP; 50 pmol of each primer; 0.75 μCi ^{35}S -dATP; and 0.5 U *Taq* DNA polymerase (Pharmacia). The reactions were amplified in a Perkin-Elmer 6900 thermal cycler, for a total of 35 cycles of 95°C for 30 s, annealing at the optimal temperature for each primer pair for 30 s (table 1), and 72°C for 30 s. The PCR products were diluted 10-fold with loading buffer (90% formamide; 10 mM EDTA, pH 8.0; 0.05% bromophenol blue; and 0.05% xylene cyanol) and were denatured at 95°C for 5 min, and then 5 μl was loaded on a 6% polyacrylamide gel containing 5% glycerol. The samples were electrophoresed at 2 W constant power, at room temperature (for the detection of the *BRCA2* mutations 2816insA and 8765delAG), or at 25 W constant power, at 4°C (for the detection of all *BRCA1* mutations and *BRCA2* mutations G6085T and 6503delTT); were transferred to Whatman paper and dried at 80°C on a vacuum gel drier; and then were autoradiographed (Hyperfilm MP, Amersham) for 12–72 h. Samples that displayed a band shift, by SSCP analysis, were PCR amplified with the appropriate SSCP primer set, and the PCR products were gel purified and sequenced by use of a U.S. Biochemical PCR product-sequencing kit, in accordance with the manufacturer's instructions.

Genotyping of 13q and 17q Markers

Polymorphic microsatellite repeat markers spanning the *BRCA1* locus (*D17S855*, *D17S1322*, *D17S1323*,

D17S1327, and *D17S1326*) (Neuhausen et al. 1996b) and the *BRCA2* locus (*D13S260*, *D13S1698*, *D13S1699*, and *D13S171*) (Couch et al. 1996) were used for haplotype analysis of families identified with mutations. The PCR conditions were as described above for the SSCP analysis, using the annealing temperatures described for the *BRCA1* (Neuhausen et al. 1996b) and *BRCA2* (Couch et al. 1996) markers. PCR products were electrophoresed on 5% acrylamide gels and were visualized as described above. The disease allele-associated haplotype was deduced by inspection of segregating genotypes in the families.

Results

Mutation Analysis

One of eight *BRCA1* or *BRCA2* mutations was identified in 41 of 97 French Canadian breast and breast/ovarian cancer families (table 2). Six of the eight mutations were observed in more than one family. The *BRCA1* C4446T and *BRCA2* 8765delAG mutations were found frequently. The *BRCA1* C2598A missense mutation, first reported in family 179 (Simard et al. 1994), was not found in the other families. Seven of the eight mutations are frameshift mutations, which are predicted to result in premature translation termination and the production of truncated proteins (table 1). The C2598A mutation, which was not detected in 86 French Canadian control individuals (Simard et al. 1994), is predicted to result in the amino acid substitution of threonine to lysine at codon 826. Because we were not able to determine whether this mutation segregates with breast cancer in family 179, the functional significance of this variant currently is unknown.

Haplotype Analysis

Haplotype analysis using polymorphic markers spanning the *BRCA1* and *BRCA2* loci was performed on index cases and, when possible, on additional family members, to determine whether carriers from different families harbored the same mutation identical by descent. Families with the same *BRCA1* mutation shared a common haplotype or a genotype compatible with a shared haplotype (table 3). The haplotype associated with each of the common *BRCA1* mutations is unique for that mutation. Although only one family has been shown to carry C2598A, the genotype of the carrier of this mutation also is distinguishable from the haplotypes of the carriers of the common *BRCA1* mutations. We were not able to phase all carriers of 8765delAG, but four haplotypes were observed in six independently ascertained families identified with this mutation, all sharing an allele of the same size for markers *D13S1698* and

Table 2
Phenotypes of French Canadian Families with *BRCA1* and *BRCA2* Mutations

		NO. OF CASES OF CANCER				
GENE AND FAMILY	MUTATION	Female Breast		Male Breast	Ovarian (Ovarian and Breast)	OTHER CANCER(S)
		At Age ≤50 Years (Bilateral)	At Age >50 Years (Bilateral)			
BRCA1:						
179	C2598A	3	1		3	Kidney, skin
290	2953del3+C	2 (1)	2		1	Kidney, ^a liver
486	2953del3+C	8 (2)	2		4 (2)	Bladder, colon, fallopian tube, kidney, leukemia, lymphoma, lung, prostate, throat
869	2953del3+C	1			3	Brain, lung, lymphoma
dp4	2953del3+C		2		3	Colon, prostate, lung
W9072	3768insA	5	1 (1)		3	
W1008A	3768insA	2 (2)			1	Stomach
151	C4446T	5 (2)			1 (1)	Colon
214	C4446T	3 (2)				Lung, papillary thyroid carcinoma ^a
252	C4446T	2	2		1	Cervical
307	C4446T	1	2		2	Brain, leukemia
476	C4446T	2	2		4	Larynx, pancreas
482	C4446T	3			1	
547	C4446T	2	1		1 (1)	Lung, sarcoma
650	C4446T	3			1	Cervical, colon, neuroblastoma
717	C4446T	4			2 (1)	Skin
767	C4446T	3	1			
813	C4446T	2			1	Throat
dp5	C4446T	3	3	1	2	Bone, colon, prostate, throat, stomach, testicular
hd92	C4446T	3			1	Colon, stomach
se2	C4446T	2	1		1 (1)	Brain, vular
so26	C4446T	4 (1)			1	
vn75	C4446T	7 (1)	1		1	Colon, lung, prostate, uterine
W9099	C4446T	2 (2)			1	Stomach
BRCA2:						
107	2816insA	5	4			Colon, liver, skin (malignant melanoma)
hd74	2816insA		3		3	Bladder, lung, stomach
719	G6085T	5		1		Prostate
hd128	G6085T	4 (1)	2			Colon, skin
ml	G6085T	3 (1)				
W9074	6503delTT	5				Brain, colon, lymphoma
616	8765delAG	2	1		2	Bone
739	8765delAG	4	1		1 (1)	Colon
825	8765delAG	5	3			Fallopian tube
853	8765delAG	3				Prostate
854	8765delAG	6	2			Stomach
hd13	8765delAG	3	1			Lung, pancreas, bone
hd86	8765delAG	5 (1)	1			Colon, leukemia, prostate
PG1940	8765delAG	12 (2)	9	2		Colon, duodenum, kidney, larynx, liver lung, lymphoma, pancreas, prostate, vater
so28	8765delAG		4		1 (1)	Esophagus, larynx, mouth, stomach
vn54	8765delAG	8 (2)	7	1		Colon, leukemia, lung, prostate, stomach, throat, uterine
W10083	8765delAG	2			1	Brain

^a Individual diagnosed with breast cancer and other cancer or cancer site named.

D13S1699 (table 4). The marker genotypes of all the carriers of 8765delAG were consistent with the possibility that they shared a common ancestry. At least two different *BRCA2* haplotypes, which differed at the proximal flanking locus, *D13S171*, were observed for

G6085T (table 4). The carriers of 2816insA shared an identical haplotype (table 4). The haplotypes of all three common *BRCA2* mutations are unique and differ from the genotype determined for the carriers of the 6503delTT mutation observed in one family.

Table 3**Haplotype Analysis of *BRCA1* Mutant-Allele Carriers for Chromosome 17q Markers**

MUTATION AND FAMILY	HAPLOTYPE, BY MARKER ^a				
	<i>D17S855</i>	<i>D17S1322</i>	<i>D17S1323</i>	<i>D17S1327</i>	<i>D17S1326</i>
C2598A:					
179	5, 6	4, 5	2, 6	7, 12	10 10
2953del3+C:					
290	11 5	4 4	6, 3	7 7	9 9
486	3 5	4 4	5 3	12 7	2 9
869	5 5	4 4	3 3	6 7	10 9
dp4	6 5	2 4	3 3	3, 7	10 9
3768insA:					
W9072	4 7	5 4	5 3	12, 5	4 10
W1008A	8, 7	5, 4	4, 3	15, 5	10 10
C4446T:					
151	6 4	4 5	2 6	5 12	10 2
214	4 4	5 5	6 6	12 12	3, 2
252	4 4	5 5	6 6	12 12	4, 2
307	7 4	5 5	6 6	2 12	10 2
476	3 4	5 5	6 6	12 12	4 2
482	2 4	5 5	6 6	12 12	4 2
547	4 4	5 5	6 6	12 12	2 2
650	2, 4	5 5	6 6	12 12	2 2
717	1, 4	5 5	4, 6	12 12	2 2
so26	1, 4	5 5	6 6	12 12	2 2
vn75	1 4	5 5	6 6	12 12	2 2
767	6 4	4 5	3 6	1 12	10 2
813	4 4	3, 5	6 6	7, 12	10, 2
hd92	6, 4	5 5	6 6	12 12	2 2
se2	6 4	3 5	6 6	4 12	10 2
dp5	4 4	5 5	6 6	12 12	2 2
W9099	6 4	4, 5	4, 6	4, 12	10, 2

^a Haplotypes segregating with *BRCA1* mutant alleles are in boldface type, haplotypes not segregating with the *BRCA1* or *BRCA2* mutant alleles are in italics, and genotypes of unphased alleles are separated by a comma.

Phenotype of Families with Mutations

The phenotypes of the families with identified mutations are shown in table 2, and the relative frequencies of mutations in breast cancer and breast/ovarian cancer families are shown in table 5. A greater proportion of families with one or more cases of ovarian cancer were found to carry a *BRCA1* mutation, rather than a *BRCA2* mutation ($P = .008$). The odds of detecting one of four *BRCA1* mutations was $18.7\times$ greater if the proband reported one or more cases of ovarian cancer in her family ($P < .00001$). Among the families with a mutation, the presence of ovarian cancer also predicted which of the two genes was found to harbor a mutation. The odds for detection of a *BRCA1* versus a *BRCA2* mutation was 26.4 when ovarian cancer was present ($P = .006$). It should be emphasized, however, that the predictive models derived from these data are based solely on the screening of four *BRCA1* and four *BRCA2* mutations.

Although our minimum inclusion criteria included at

least one case of breast cancer diagnosed at <65 years of age, families harboring a *BRCA2* mutation contained, on average, 6.7 cases of breast cancer, in contrast to families harboring one of four *BRCA1* mutations (3.9 cases/family) and to families found not to harbor any of the eight mutations (3.7 cases/family). Among the 97 families, the odds of finding one of four *BRCA2* mutations was 5.3 if five or more cases of breast cancer were present ($P = .003$). Among the subset of 41 families in which one of eight mutations was detected, the odds of finding one of the four *BRCA2* mutations (versus one of the four *BRCA1* mutations) was 5.4 if five or more cases of breast cancer were present ($P = .02$).

The average age at breast cancer diagnosis was younger in families harboring one of the four *BRCA1* mutations (43.4 years) than in families not harboring a *BRCA1* mutation (48.9 years) ($P = .0002$). The average age at breast cancer diagnosis also was younger in families harboring one of the four *BRCA2* mutations (46.8 years) than in families not harboring a *BRCA2* muta-

Table 4

Haplotype Analysis of *BRCA2* Mutant-Allele Carriers for Chromosome 13q Markers

MUTATION AND FAMILY	HAPLOTYPE, BY MARKER ^a			
	D13S260	D13S1698	D13S1699	D13S171
2816insA:				
107	4 7	12 11	3 4	9 2
hd74	8 7	3 11	1 4	9 2
G608ST:				
719	2 6	9 9	4, 3	9 9
hd128	7, 6	9 9	4 3	7 9
ml	7, 6	9 9	3 3	7 7
6503delTT:				
W9074	7, 9	8, 9	3, 4	9 9
8765delAG:				
616	9 4	9 11	4 3	9 9
739	7 4	9, 11	3 3	9 9
825	5, 4	9, 11	4, 3	2, 9
853	4 4	9, 11	4, 3	2, 9
854	8, 4	11 11	3 3	2, 9
hd13	4 7	12 11	3 3	2 9
hd86	4 7	11 11	3 3	2 2
PG1940	5 4	11 11	3 3	2 9
so28	7 7	9 11	3 3	9 9
vn54	7 4	9 11	4 3	2 9
W10083	4, 7	9, 11	3 3	7, 9

^a Haplotypes segregating with a *BRCA2* mutation are indicated in boldface type, and genotypes of unphased alleles are separated by a comma.

tion, but the difference was not significant ($P = .17$). In Canada, the average age at diagnosis of ovarian cancer is 56 years. The average age at diagnosis of ovarian cancer for carriers of one of the four *BRCA1* mutations was 50.6 years, and the average age at diagnosis for carriers of one of the four *BRCA2* mutations was 59.6 years. Although the average age at diagnosis for *BRCA1* carriers was slightly younger than that for noncarriers ($P = .55$), the average age at diagnosis for *BRCA2* carriers was older than those for noncarriers ($P = .09$) and *BRCA1* carriers ($P = .02$).

Although the selection criteria included the number of female breast cancer cases diagnosed at <65 years of age or the number of cases of ovarian cancer, a significant proportion of families found to harbor a mutation contained at least one breast cancer case diagnosed at <36 years of age. Twenty-six (58%) of 45 families with a case of breast cancer diagnosed at <36 years of age carried a mutation, compared with only 15 (29%) of 52 families with no case of breast cancer before 36 years of age (odds ratio 3.4; $P = .004$). In addition, among the families with no ovarian cancer cases, the frequency of mutations increased for families containing more than one breast cancer case diagnosed before 36 years of age ($P = .0012$). Mutations were present in 8%, 33%, 67%, and 80% of families with totals of zero, one, two, or three or more (maximum of seven) cases, respectively.

Discussion

French Canadian families containing at least three female breast cancer cases (<65 years of age), ovarian cancer, or male breast cancer were screened for four *BRCA1* and four *BRCA2* mutations. A mutation was identified in 41 of 97 families: 24 families had a *BRCA1* mutation, and 17 families had a *BRCA2* mutation. Six of the eight mutations were identified in 40% of the families and accounted for 95% of mutations identified in families with mutations. The *BRCA1* C4446T and *BRCA2* 8765delAG mutations accounted for the majority of mutations identified in the French Canadian cancer families. Although direct comparisons are difficult because of the differences in selection criteria and because a partial screen was performed, the proportion of French Canadian cancer families identified to have mutations is within the range observed in other studies that used a comprehensive screen of *BRCA1* and/or *BRCA2* (reviewed in Szabo and King 1997).

Although the predictive models used in this study were based solely on the screening of four *BRCA1* and four *BRCA2* mutations in French Canadian cancer families, as has been observed elsewhere, *BRCA1* mutations were more likely to be identified in families with one or more cases of ovarian cancer, and *BRCA2* mutations were more likely to be identified in families with multiple cases of female and male breast cancer (Narod et al. 1995; Tonin et al. 1996; Shattuck-Eidens et al. 1997; Szabo and King 1997). Historically, French Canadian families typically have been large, and therefore the likelihood of identifying multiple cases of breast and ovarian cancer perhaps may be increased. In addition, previous studies have also described carriers of mutations in the breast cancer-susceptibility genes who do not have a strong family history of breast and/or ovarian cancer (Tonin et al. 1996; Levy-Lahad et al. 1997; Stratton et al. 1997).

One individual in each of the two families (486 and 825) in our series was diagnosed with fallopian tube cancer, and in both cases a mutation was identified. Cases of fallopian tube cancer have been reported in individuals who were found to be carriers of germ-line mutations (Simard et al. 1994; Tonin et al. 1996; Schubert et al. 1997). Although in this study the association of fallopian tube cancers and gene carriers is not statistically significant ($P = .12$), the pathological similarity to ovarian cancers and the familial association with other primary cancers, such as breast cancer (Podratz et al. 1986; Simard et al. 1994; Tonin et al. 1996; Schubert et al. 1997), warrant further study, to determine whether germ-line mutations in the breast cancer-susceptibility genes confer an increased risk for fallopian tube cancers.

Carriers of the most common mutations (C4446T and 8765delAG) in the French Canadian families were found

Table 5**Frequencies of Breast and Ovarian Cancer Cases and of *BRCA1* and *BRCA2* Mutations, in French Canadian Families**

No. of Female Breast Cancers/Family	No. of Ovarian Cancers/Family	Total No. of Families (No. of Families with Male Breast Cancer) [n = 97]	Total No. (%) of Families with Mutations [n = 41]	No. of Families with a <i>BRCA1</i> Mutation [n = 24]	No. of Families with a <i>BRCA2</i> Mutation [n = 17]
2	0	1 (1)	0	0	0
3	0	15 (1)	3 (20)	1	2
4	0	12	2 (17)	1	1
5+	0	20 (4)	9 (45)	0	9
2	1	12	4 (33)	3	1
3	1	10 (1)	5 (50)	5	0
4	1	6	4 (67)	3	1
5+	1	5	3 (60)	2	1
1	2+	3	1 (33)	1	0
2	2+	3	1 (33)	1	0
3	2+	4	3 (75)	1	2
4	2+	3	3 (100)	3	0
5+	2+	3 (1)	3 (100)	3	0

to share identical or similar haplotypes, providing evidence that disease alleles are identical by descent. The birthplaces of the grandparents of the mutation carriers were determined (fig. 1). The birthplaces of the grandparents of the carriers with the most common mutations overlap and have been found predominantly in the southern part of the province of Quebec. The founders who settled in Quebec before 1680 have been estimated to account for approximately two-thirds of the present French Canadian gene pool in Quebec (Charbonneau et al. 1987). This geographic region of early settlement overlaps the areas in which families with the most common mutations have been found (fig. 1). The two most common mutations are likely to have been introduced early in the settlement of Quebec. Carriers of the less common mutations, which appear to be identical by descent, by haplotype analysis, originated from different geographic regions within Quebec (fig. 1). The founders did not contribute uniformly to the contemporary gene pool, since the contribution observed for some of them is, in part, the result of a high reproduction rate, compared with that of later immigrants, and also of a different migration pattern (Heyer and Trembley 1995; Labuda et al. 1997). In addition, the possibility that more recent immigrants (after 1870) were also carriers of the same disorders but had different mutations cannot be ruled out.

Some of the mutations reported in the French Canadian population have been reported in other independent studies. The C4446T mutation is one of the most common *BRCA1* mutations found in the Breast Cancer Information Core database, and haplotype analysis has suggested that this mutation may have arisen independently at least three times (Neuhausen et al. 1996b). The

haplotype segregating with the C4446T mutation in the French Canadian families is comparable to that segregating in the two American families reported by Neuhausen et al. (1996b). More recently, this mutation was also identified in two families in France (Stoppa-Lyonnet et al. 1997). The *BRCA2* G6085T mutation has been reported previously in families from North America (Phelan et al. 1996) and France (Serova-Sinilnikova et al. 1997). The genealogy of one of the French Canadian families carrying the G6085T mutation has revealed origins in the provinces of Aunis and Normandy, in France. This finding was not surprising, since the majority of early settlers in Quebec emigrated from these two provinces in France (Charbonneau and Robert 1987). The *BRCA2* 6503delTT mutation has been reported in families in England, and British families that carry this mutation have been shown to share intragenic polymorphisms (Mazoyer et al. 1996). This *BRCA2* mutation may have been introduced by a carrier who was of British descent, a likely possibility given that the early settlers in Quebec originated from France and England (Charbonneau and Robert 1987). It would be interesting to determine whether carriers of mutations found in the French Canadian cancer families shared a common ancestry with other families shown to carry identical mutations.

Variation in risk of breast and ovarian cancer have been associated with germ-line *BRCA2* mutations (Gayther et al. 1997). The analysis of the mutation distribution along the length of the gene indicated a significant genotype-phenotype correlation, with mutations causing the highest risk of ovarian cancer relative to breast cancer clustered in a region of ~3.3 kb in exon 11 (Gayther et al. 1997). Five of the 17 French Canadian families

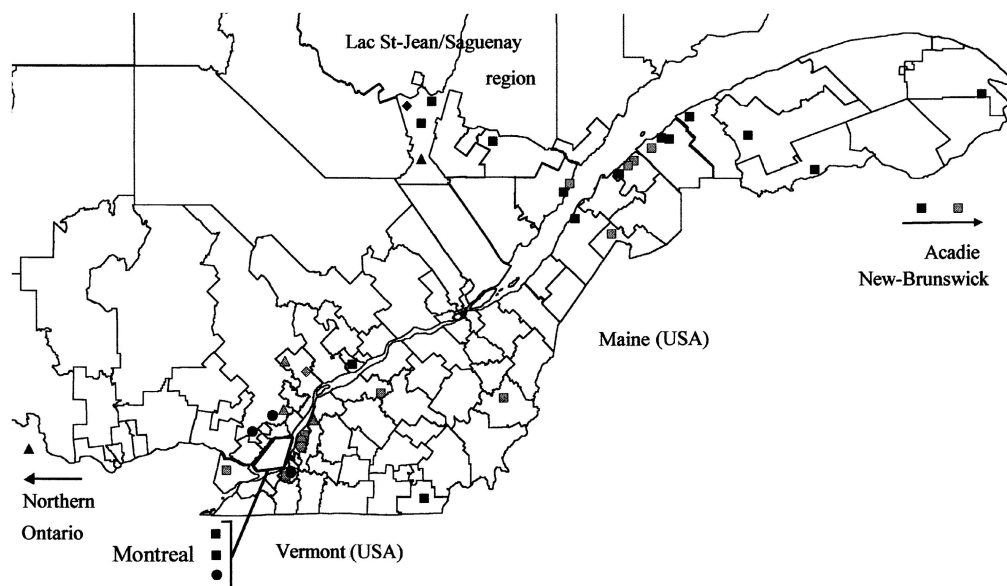


Figure 1 Population distribution of *BRCA1* and *BRCA2* mutations identified in the French Canadian population. Only the southern region of the province of Quebec, including the Lac St. Jean/Saguenay region and the territory surrounding the St. Lawrence River, is depicted. Each symbol indicates the location of the grandparental generation of the families that have the *BRCA1* mutations C4446T (blackened squares), 2953del3+C (blackened circles), 3768insA (blackened triangles), and C2598A (blackened diamonds) and those that have the *BRCA2* mutations 8765delAG (grey-shaded squares), 2816insA (grey-shaded circles), G6085T (grey-shaded triangles), and 6503delTT (grey-shaded diamonds). The arrows with a symbol(s) indicate the location in northern Ontario and in New Brunswick of families with mutations. The divisions within the province represent catchment areas for each health care center (Centre Local de Services Communautaires).

carrying a *BRCA2* mutation had at least one ovarian cancer case. Two of the three common *BRCA2* mutations found in these families occurred outside this “ovarian cancer cluster” region. None of the three families carrying the G6085T mutation, which is located in the ovarian cancer cluster region, included cases of ovarian cancer. Mutations in the distal 3' end of the *BRCA2* gene in families with breast and ovarian cancer also have been reported (Hakansson et al. 1997; Serova-Sinilnikova et al. 1997; Vehmanen et al. 1997).

The French Canadian cancer families examined in this study were recruited from gynecology and oncology, breast cancer, and hereditary-cancer clinics and thus represent a cross section of families that may seek genetic counseling. Thus, the identification of common mutations in French Canadian breast and ovarian cancer families has important consequences for genetic testing. Although additional mutations may account for a proportion of the 56 families found not to carry one of the eight mutations in the screen, a complete genomic screen of *BRCA1* and *BRCA2* in the index cases from 14 of these families (which contained three or more cases of breast and/or ovarian cancer) has failed to reveal a mutation in either gene (authors' unpublished data). The frequency of mutations identified in the French Canadian cancer families is the same as the frequency of mu-

tations found in a recent examination of 220 Ashkenazi Jewish families (Tonin et al. 1996). However, unlike the situation for Ashkenazi families, mutations in the *BRCA2* gene accounted for the majority of French Canadian breast cancer families found to harbor mutations. Overall, 57% of French Canadian breast/ovarian cancer families with at least two breast cancer cases and one ovarian cancer case were found to have a mutation, compared with 73% of Ashkenazi breast/ovarian cancer families. Mutations were found in a large majority of both French Canadian and Ashkenazi Jewish families with at least two cases each of breast cancer and ovarian cancer. In addition, mutations in *BRCA1* were found in the majority of breast/ovarian cancer families, in both ethnic groups. However, in contrast to the Ashkenazi Jewish population, in which the prevalence of carriers of *BRCA1* and *BRCA2* mutations is known to be high, ~2.5% (Struwing et al. 1995a; Oddoux et al. 1996; Roa et al. 1996), the overall prevalence of carriers of the mutations identified in the French Canadian population is not yet known.

Acknowledgments

We wish to thank the participants and their families for their cooperation; research technicians France Dion, Marie-Claude

Faucher, Andrew Manning, Louise Champoux, Maria Galvez, and Pauline Rehal; research associates Chantal Perret and Beatrice Godard; genetic counselors Lidia Kasprzak, Corinne Seruya, Jennifer Ozaki, Nora Wong, Ophira Ginsburg, Shari Miller, and Betty Wong; Drs. Pierre Drouin, Josée Dubuc-Lissoir, Philippe Gauthier, Pierre Audet-Lapointe, David Rosenblatt, and Harvey Risch. This work was supported by grants from the Canadian Breast Cancer Foundation (to P.N.T., A.-M.M.-M., D.P., and S.A.N.), Canadian Genetic Diseases Network (Network of Centres of Excellence Program) (to K.M. and S.A.N.), Fonds de Research en Santé du Québec (Fonds de la Recherche en Santé du Québec–Family Cancer Network) (to W.D.F., P.G., A.-M.M.-M., D.P., and P.N.T.), National Cancer Institute of Canada (to P.G., P.N.T., W.D.F., and S.A.N.), and National Cancer Institute/Duke University Specialist Program of Research Excellence in Breast Cancer (to P.A.F.). A.-M.M.-M. is the recipient of an FRSQ senior fellowship, and P.N.T. is a Medical Research Council of Canada–Cancer Research Society, Inc., scholar.

Electronic-Database Information

The URL for data in this article is as follows:

Breast Cancer Information Core, http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic

References

- Berman DB, Costals J, Schultz DC, Grana G, Daly M, Godwin AK (1996a) A common mutation in *BRCA2* that predisposes to a variety of cancers is found in both Jewish Ashkenazi and non-Jewish individuals. *Cancer Res* 56:3409–3414
- Berman DB, Wagner-Costalas J, Schultz DC, Lynch HT, Daly M, Godwin AK (1996b) Two distinct origins of a common *BRCA1* mutation in breast-ovarian cancer families: a genetic study of 15 185delAG mutation kindreds. *Am J Hum Genet* 58:1166–1176
- Bouchard G, Laberge C, Scriver CR (1988) Reproduction démographique et transmission génétique dans le nord-est de la province de Québec (18e–20e siècles). *Eur J Popul* 4: 39–67
- Brais B, Xie Y-G, Sanson M, Morgan K, Weissenbach J, Korczyn AD, Blumen SC, et al (1995) The oculopharyngeal muscular dystrophy locus maps to the region of the cardiac α and β myosin heavy chain genes on chromosome 14q11.2-q13. *Hum Mol Genet* 4:429–434
- Casabon LK, Melanson M, Lopes-Cendes I, Maréchal C, Andermann E, Andermann F, Weissenbach J, et al (1996) The gene responsible for a severe form of peripheral neuropathy and agenesis of the corpus callosum maps to chromosome 15q. *Am J Hum Genet* 58:28–34
- Charbonneau H, Desjardins B, Guillemette A, Landry Y, Légaré J, Nault F (1987) Naissance d'une population: les Français établis au Canada au XVIIIe siècle. Presse de l'Université de Montréal, Montréal
- Charbonneau H, Robert N (1987) The French origins of the Canadian population 1608–1759. In: Harris RC (ed) From the beginning to 1800. Vol 1 in: Historical atlas of Canada. University of Toronto Press, Toronto, plate 45
- Couch FJ, Rommens JM, Neuhausen SL, Bélanger C, Dumont M, Abel K, Russell B, et al (1996) Generation of an integrated transcription map of the *BRCA2* region on chromosome 13q12-q13. *Genomics* 36:86–99
- Davignon J, Roy M (1993) Familial hypercholesterolemia in French-Canadians: taking advantage of the presence of a “founder effect. *Am J Cardiol* 72:6D–10D
- De Braekeleer M, Dao TN (1993) Founder effect in beta-thalassaemia in Portneuf, Quebec. *Nouv Rev Fr Hematol* 35: 447–450
- (1994a) Hereditary disorders in the French Canadian population of Quebec. I. In search of founders. *Hum Biol* 66:205–223
- (1994b) Hereditary disorders in the French Canadian population of Quebec. II. Contributions of Perche. *Hum Biol* 66:225–249
- Durocher F, Tonin P, Shattuck-Eidens D, Skolnick M, Narod SA, Simard J (1996) Mutation analysis of *BRCA1* gene in 23 families with cases of cancer of the breast, ovary, and multiple other sites. *J Med Genet* 33:814–819
- Fitzgerald MG, MacDonald DJ, Krainer M, Hoover I, O'Neil E, Unsal H, Silva-Arrieto S, et al (1996) Germline *BRCA1* mutations in Jewish and non-Jewish women with early-onset breast cancer. *N Engl J Med* 334:143–149
- Friedman L, Ostermeyer E, Szabo C, Dowd P, Butler L, Park T, Lee MK, et al (1995) Novel inherited mutations and variable expressivity of *BRCA1* alleles, including the founder mutation 185delAG in Ashkenazi Jewish families. *Am J Hum Genet* 57:1284–1297
- Gayther SA, Harrington P, Russell P, Kharkevich G, Garkavtseva RF, Ponder BAJ (1997) Frequently occurring germ-line mutations of the *BRCA1* gene in ovarian cancer families from Russia. *Am J Hum Genet* 60:1239–1242
- Gayther SA, Warren W, Mazoyer S, Russell PA, Harrington PA, Chiano M, Seal S, et al (1995) Germline mutations of the *BRCA1* gene in breast and ovarian cancer families provide evidence for phenotype-genotype correlations. *Nat Genet* 11:428–433
- Hakansson S, Johannsson O, Johannsson U, Selberg G, Loman N, Gerdes A-M, Holmberg E, et al (1997) Moderate frequency of *BRCA1* and *BRCA2* germ-line mutations in Scandinavian familial breast cancer. *Am J Hum Genet* 60: 1068–1078
- Heyer E, Trembley M (1995) Variability of the genetic contribution of Quebec population founders to some deleterious genes. *Am J Hum Genet* 56:970–978
- Hogervorst FB, Cornelis RS, Bout M, van Vliet M, Oosterwijk JC, Olmer R, Bakker B, et al (1995) Rapid detection of *BRCA1* mutations by the protein truncation test. *Nat Genet* 10:208–212
- Johannesdottir G, Gudmundsson J, Bergthorsson JT, Arason A, Eikiksdottir G, Johannsson OT, Borg A, et al (1996) High prevalence of the 999del5 mutation in Icelandic breast and ovarian cancer patients. *Cancer Res* 56:3663–3665
- Johannsson O, Ostermeyer EA, Hakansson S, Friedman LS, Johannsson U, Sellberg G, Brondum-Nielsen K, et al (1996) Founding *BRCA1* mutations in hereditary breast and ovar-

- ian cancer in southern Sweden. *Am J Hum Genet* 58: 441-450
- Labuda D, Zietkiewicz E, Labuda M (1997) The genetic clock and the age of the founder effect in growing populations: a lesson from French Canadians and Ashkenazim. *Am J Hum Genet* 61:768-771
- Labuda M, Labuda D, Korablaskoska M, Cole DEC, Zeitkiewicz E, Weissenbach J, Popowska E, et al (1996) Vitamin D deficiency, rickets, and the founder effect in French Canadians. *Am J Hum Genet* 59:633-643
- Levy-Lahad E, Catane R, Eisenberg S, Kaufman B, Hornreich G, Lishinsky E, Shohat M, et al (1997) Founder BRCA1 and BRCA2 mutations in Ashkenazi Jews in Israel: frequency and differential penetrance in ovarian cancer and in breast-ovarian cancer families. *Am J Hum Genet* 60: 1059-1067
- Mazoyer S, Dunning AM, Serova O, Dearden J, Puget N, Healey CS, Gayther SA, et al (1996) A polymorphic stop codon in BRCA2. *Nat Genet* 14:253-254
- Miki Y, Swenson J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Lui Q, et al (1994) A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* 266:66-71
- Morin C, Mitchell G, Larochelle J, Lambert M, Ogier H, Robinson BH, De Braekeleer M (1993) Clinical, metabolic, and genetic aspects of cytochrome C oxidase deficiency in Saguenay-Lac-Saint-Jean. *Am J Hum Genet* 53:488-96
- Narod SA, Ford D, Devilee P, Barkardottir RB, Lynch HT, Smith SA, Ponder BAJ, et al (1995) An evaluation of genetic heterogeneity in 145 breast-ovarian cancer families. *Am J Hum Genet* 56:254-264
- Neuhausen S, Gilwesi T, Norton L, Tran T, McGuire P, Swensen J, Hempel H, et al (1996a) Recurrent BRCA2 6174delT mutations in Ashkenazi Jewish women affected by breast cancer. *Nat Genet* 13:126-128
- Neuhausen SL, Mazoyer S, Friedman L, Stratton M, Offit K, Caligo A, Tomlinson G, et al (1996b) Haplotype and phenotype analysis of six recurrent BRCA1 mutations in 61 families: results of an international study. *Am J Hum Genet* 58:271-280
- Oddoux C, Struwing JP, Clayton CM, Neuhausen S, Brody LC, Kaback M, Haas B, et al (1996) The carrier frequency of the BRCA2 6174delT mutation among Ashkenazi Jewish individuals is approximately 1%. *Nat Genet* 14:188-190
- Peelen T, van Vliet M, Petrij-Bosch A, Mieremet R, Szabo C, van den Ouweland AMW, Hogervost F, et al (1997) A high proportion of novel mutations in BRCA1 with strong founder effects among Dutch and Belgian hereditary breast and ovarian cancer families. *Am J Hum Genet* 60:1041-1049
- Phelan C, Lancaster J, Tonin P, Gumbs C, Cochran C, Carter R, Ghadirian P, et al (1996) Mutation analysis of 49 site-specific breast cancer families. *Nat Genet* 13:120-122
- Podratz KC, Podczaski ES, Gaffey TA, O'Brien PC, Schray MF, Malkasian GD Jr (1986) Primary carcinoma of the fallopian tube. *Am J Obstet Gynecol* 154:1319-1326
- Ramus SJ, Kote-Jarai Z, Friedman LS, van der Looij M, Gathayer SM, Csokay B, Ponder BAJ, et al (1997) Analysis of BRCA1 and BRCA2 mutations in Hungarian families with breast or breast-ovarian cancer. *Am J Hum Genet* 60: 1242-1246
- Roa BB, Boyd AA, Volcik K, Richards CS (1996) Ashkenazi Jewish population frequencies for common mutations in BRCA1 and BRCA2. *Nat Genet* 14:185-187
- Rozen R, De Braekeleer M, Daigneault J, Ferreira-Rajabi L, Gerdes M, Lamoureux L, Aubin G, et al (1992) Cystic fibrosis mutations in French Canadians: three CFTR mutations are relatively frequent in a Quebec population with an elevated incidence of cystic fibrosis. *Am J Med Genet* 42: 360-364
- Schubert EL, Lee MK, Mefford HC, Argonza RH, Morrow JE, Hull J, Dann JL, et al (1997) BRCA2 in American families with four or more cases of breast or ovarian cancer: recurrent and novel mutations, variable expression, penetrance, and the possibility of families whose cancer is not attributable to BRCA1 and BRCA2. *Am J Hum Genet* 60: 1031-1040
- Serova O, Montagna M, Torchard D, Narod SA, Tonin P, Sylla B, Lynch HT, et al (1996) A high incidence of BRCA1 mutations in 20 breast-ovarian cancer families. *Am J Hum Genet* 58:42-51
- Serova-Sinilnikova OM, Boutrand L, Stoppa-Lyonnet D, Bresac-de-Paillerets B, Dubois V, Lasset C, Janin N, et al (1997) BRCA2 mutations in hereditary breast and ovarian cancer in France. *Am J Hum Genet* 60:1236-1239
- Shattuck-Eidens D, McClure M, Simard J, Labrie F, Narod S, Couch F, Hoskins K, et al (1995) A collaborative survey of 80 mutations in the BRCA1 breast and ovarian cancer susceptibility gene: implications for presymptomatic testing and screening. *JAMA* 273:535-541
- Shattuck-Eidens D, Oliphant A, McClure M, McBride C, Gupta J, Rubano T, Pruss D, et al (1997) BRCA1 sequence analysis in women at high risk for susceptibility mutations: risk factor analysis and implications for genetic testing. *JAMA* 278:1242-1250
- Simard J, Tonin P, Durocher F, Morgan K, Rommens J, Gingras S, Samson C, et al (1994) Common origins of BRCA1 mutations in Canadian breast and ovarian cancer families. *Nat Genet* 8:392-398
- Stajich JM, Gilchrist JM, Lennon F, Lee A, Yamaoka L, Helms B, Gaskell PC, et al (1996) Confirmation of linkage of oculopharyngeal muscular dystrophy to chromosome 14q11.2-q13. *Ann Neurol* 40:801-804
- Stoppa-Lyonnet D, Laurent-Puig P, Essioux L, Pagès S, Ithier G, Ligtot L, Fourquet A, et al (1997) BRCA1 sequence variations in 160 individuals referred to a breast/ovarian family cancer clinic. *Am J Hum Genet* 60:1021-1030
- Stratton JF, Gayther SA, Russell P, Dearden J, Gore M, Blake P, Easton D, et al (1997) Contribution of BRCA1 mutations to ovarian cancer. *N Engl J Med* 336:1125-1130
- Struwing JP, Abeliovich D, Peretz T, Avishai N, Kaback MM, Collins FS, Brody LC (1995a) The carrier frequency of the BRCA1 185delAG mutation is approximately 1 percent in Ashkenazi Jewish individuals. *Nat Genet* 11:198-200
- Struwing JP, Brody LC, Erdos MR, Kae RG, Giambarrisi TR, Smith SA, Collins FS, et al (1995b) Detection of eight BRCA1 mutations in 10 breast/ovarian cancer families, including 1 family with male breast cancer. *Am J Hum Genet* 57:1-7
- Szabo CI, King M-C (1997) Population genetics of BRCA1 and BRCA2. *Am J Hum Genet* 60:1013-1020

- Tavitgian SV, Simard J, Rommens J, Couch F, Shattuck-Eidens D, Neuhausen S, Merajver S, et al (1996) The complete *BRCA2* gene and mutations in chromosome 13q-linked kindreds. *Nat Genet* 12:333–337
- Tonin P, Serova O, Lenoir G, Lynch H, Durocher F, Simard J, Morgan K, et al (1995) *BRCA1* mutations in Ashkenazi Jewish women. *Am J Hum Genet* 57:189
- Tonin P, Weber B, Offit K, Couch F, Rebbeck TR, Neuhausen S, Godwin AK, et al (1996) Frequency of recurrent *BRCA1* and *BRCA2* mutations in Ashkenazi Jewish breast cancer families. *Nat Med* 2:1179–1183
- Vehmanen P, Friedman LS, Eerola H, Sarantaus L, Pyrhonen S, Ponder BAJ, Muhonen T, et al (1997) A low proportion of *BRCA2* mutations in Finnish breast cancer families. *Am J Hum Genet* 60:1050–1058
- Wagner TMU, Moslinger R, Zielinski C, Scheiner O, Brieteneder H (1996) New Austrian mutation in *BRCA1* gene detected in three unrelated HBOC families. *Lancet* 347:1263
- Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, et al (1995) Identification of the breast cancer susceptibility gene *BRCA2*. *Nature* 378:789–792